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Serum Neopterin, β_2-Microglobulin, Soluble Interleukin-2 Receptors, and Immunoglobulin Levels in Healthy Adolescents

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Serum biomarkers, such as neopterin, β_2-microglobulin (B2M), and soluble interleukin-2 receptors (sIL-2R), are elevated in viral infections, including HIV-1 infection, and in inflammatory conditions, autoimmune disease, and malignancies. For many of these conditions, serum levels correlate with disease activity. Application of these biomarkers in adolescents is limited by a lack of information on the range and determinants of variability (age, sex, race) for serum levels of these important molecules in this age group. To address this question, we analyzed serum samples from a well-characterized heterogeneous population of 111 healthy adolescents. White children had significantly higher serum levels of sIL-2R and IgM and lower levels of IgG ($P \leq 0.001$) than black children. Boys had higher sIL-2R and B2M levels ($P < 0.005$) and lower IgM levels ($P < 0.05$) than girls. No significant age effect on B2M or neopterin level was observed over the age range of 12–19 years included in this analysis. However, stratification by race showed that serum sIL-2R level was significantly associated with age among whites, but not among blacks. Values of these biomarkers in this population are compared with age-stratified values in the previously analyzed 20- to 69-year-old population from whose households the adolescent subjects were recruited.

Key Words: **neopterin; β_2-microglobulin; soluble interleukin-2 receptors; age; race; gender.**

INTRODUCTION

Neopterin, β_2-microglobulin (B2M), and soluble interleukin-2 receptors (sIL-2R) have been used as markers of immune activation. Neopterin is produced by macrophages through a cascade of cytokine-stimulated enzymatic reactions which ultimately result in conversion of guanosine triphosphate into neopterin. Serum levels are believed to reflect activation of these phagocytes and are elevated in patients with neoplastic disease, immune disease, infections, and transplants (1). In subjects with viral and parasitic infections and autoimmune disease, serum and urine neopterin levels are elevated, and fluctuations correlate with disease activity (2). Neopterin levels also increase significantly with solid organ transplant rejection (2, 3). Pretransplant urinary neopterin levels were predictive of mortality risk and were correlated with tumor stage in a study of women with ovarian cancer (4). Serum B2M levels are believed to reflect general immune activation and lymphoid cell turnover and, like neopterin levels, are elevated in viral infections, inflammatory conditions, autoimmune disease, and malignancy (5, 6).

sIL-2R is a cleavage product of the membrane-bound IL-2 receptor and is released from activated lymphocytes. By competing with cellular IL-2R for IL-2 binding, sIL-2R is believed to play a role in locally downregulating immune responses (7, 8). Serum levels may provide a measure of systemic immune activation. Serum sIL-2R levels are elevated in patients with rheumatoid arthritis and systemic lupus erythematosus, and high levels correlate with disease activity (9). In a study of severe atopic eczema, serum sIL-2R levels were significantly elevated in patients compared to controls, and levels correlated significantly ($R = 0.73$) with clinical disease activity (10). Serum sIL-2R levels increase significantly in rejection episodes among solid organ transplant patients (11, 12). sIL-2R levels are also elevated in patients with Wegener's granulomatosis; levels correlate with disease activity and may be an

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early marker of relapse among patients in clinical remission after treatment (13).

Serum levels of these biomarkers, as well as immunoglobulin-A (IgA), are also elevated in HIV-1-infected individuals, and the degree of elevation is related to the stage of infection (14–22). As investigations of disease processes, including HIV-related diseases, increasingly focus on preclinical disease, there is a need for basic information on these markers in healthy as well as diseased subjects. We have previously analyzed serum levels of these markers in a population-based stratified random sample of healthy adults to establish expected ranges of normality and determinants of variability (23–25). Clinical research into conditions which affect adolescents, such as autoimmune disease, viral and parasitic infections, and transplant rejection may benefit from an understanding of the relationships between these biomarkers and important host characteristics such as age, race, and gender. Acquired immunodeficiency syndrome, in particular, has become an increasingly important cause of death among adolescents (26). The current analysis was undertaken to investigate the range and determinants of variability (age, sex, race) for these important biomarkers in a population of healthy adolescents.

METHODS

Study Population

Teenagers for this investigation were enrolled from households participating in a large population-based survey of healthy adults. Selection of the households and children has been described in detail elsewhere (27, 28). Briefly, random-digit dialing was utilized to select a population-based random sample of adult subjects in the Washington, DC, metropolitan area. Demographic, life-style, and medical information was collected through telephone and self-administered questionnaires. Approximately one-third of potential adult study subjects were excluded on the basis of life-style characteristics (intravenous drug use) or medical conditions (blood transfusion after 1975, recent hospitalization, severe allergies, use of steroid medications, history of connective tissue disease, or recent pregnancy) which might affect the immunologic parameters under investigation. Racial groups other than whites and blacks were excluded due to the small number of subjects predicted from census data. Participating adults with children between the ages of 12 and 19 were asked to enroll 1 or 2 children from the household in the present study. Each of these children completed a detailed medical history questionnaire at the time of phlebotomy. All questionnaire data were reviewed by study investigators, and all children were considered healthy. Two children reported asthma; supplementary analysis excluding these subjects did not significantly alter the results. From the 374 households included in the adult study, 112 children from 83 households participated in the investigation. One child had incomplete laboratory data and was excluded from the present analysis, leaving a final study population of 111 subjects.

Sample Preparation

Blood for serum samples was collected in evacuated tubes and allowed to clot. The serum was separated by centrifugation, divided into 1.0-ml aliquots, frozen, and stored in a liquid nitrogen freezer until withdrawn for analysis.

sIL-2R Assay

Serum sIL-2R concentrations were measured by a sandwich ELISA assay developed by Rubin *et al.* (7). Briefly, alternate columns of the inner 60 wells of microtiter plates were coated with anti-Tac antibody in buffer. After incubation at 4°C overnight, the plates were washed and 100 μl of sample was added to coated and control wells. After a 2-h incubation at room temperature, the plates were washed, and FITC-labeled 7G7B6 monoclonal antibody was added to each well. After a second 2-h incubation at room temperature, the plates were again washed, and 100 μl of alkaline phosphatase-conjugated rabbit anti-FITC was added. After an additional 1-h incubation at room temperature, *p*-nitrophenyl phosphate (Sigma) was added, and the absorbance was measured at 405 nm. The absorbance of the control wells was subtracted from the experimental wells, and the absorbance value was compared to absorbance determined for a standard curve generated for each plate from known concentrations of sIL-2R.

Serum B2M and Neopterin Assay

Serum B2M was measured using a commercial double-antibody radioimmunoassay (Beta-2-Micro RIA; Pharmacia, Uppsala, Sweden). Neopterin was measured by a commercial radioimmunoassay (Neopterin RIAcid; Henning-Berlin, Berlin).

Immunoglobulin Analysis

Serum IgG, IgA, and IgM were measured by a commercial laboratory (Metpath, Inc., Rockville, MD) using nephelometry (IgG, IgA, IgM).

TABLE 1
Age, Race, and Gender Distribution of Study Subjects

	Age group (years)					
	12–14		15–16		17–18	
	Boys	Girls	Boys	Girls	Boys	Girls
White	11	20	12	17	16	7
Black	6	1	6	6	7	2
Total	17	21	18	23	23	9

Statistical Analysis

Statistical analyses were performed using the PC–SAS version 6.04 statistical analysis package (SAS Institute, Cary, NC). A Gaussian distribution for each marker was approximated with a log-transformation. The geometric (transformed) means and standard errors of each marker are presented for each of the factors examined. Testing for statistical differences in each marker by age, race, and gender was accomplished by linear regression analysis with statistical significance defined at the $P = 0.05$ level.

RESULTS

Demographic Characteristics

The study population consisted of 111 teenagers ages 12–19 years (Table 1). Females constituted 48% and blacks 25% of the study sample. Study subjects were categorized into three age groups to approximate developmental stages: 12–14 years (mostly prepubertal; $n = 38$), 15–16 years (pubertal; $n = 41$), and 17–19 years (mostly postpubertal; $n = 32$).

Distributions of Serum Levels of sIL-2R, B2M, Neopterin, and Immunoglobulins

The distributions of sIL-2R, B2M, neopterin, IgA, IgG, and IgM values among all subjects were log-transformed to approximate a Gaussian distribution; there were no missing data. The geometric means (±SE) for these biomarkers in this population were SIL-2R 559 ± 30 U/ml, B2M 1.17 ± 0.03 mg/L, neopterin 4.42 ± 0.11 nmol/L, IgA 182 ± 7 mg/dl, IgG 1279 ± 24 mg/dl, and IgM 164 ± 8 mg/dl. The range of each marker was SIL-2R 105–2270 U/ml, B2M 0.7–2.4 mg/L, neopterin 2.2–9.1 nmol/L, IgA 72–394 mg/dl, IgG 477–2320 mg/dl, and IgM 440–739 mg/dl.

Influence of Race and Gender on Serum sIL-2R, B2M, Neopterin, and Immunoglobulin Levels

For sIL-2R, B2M, and neopterin, differences by race and gender reflected previously observed associations among adults in the larger population, although some were not statistically significant (Table 2). Whites had significantly higher serum levels of sIL-2R and borderline significantly higher levels of B2M and neopterin than blacks. Whites had significantly lower levels of IgG and higher levels of IgM than blacks. Boys had

TABLE 2
Influence of Race and Gender on Serum sIL-2R, B2M, Neopterin, and Immunoglobulin Levels

	Previous observation in adults (reference)	Observation in healthy adolescents (GM ± SE)		
		Blacks	Whites	*P* value[a]
sIL-2R (U/ml)	B < W (23)	423 ± 30	615 ± 38	0.0004
B2M (mg/L)	B < W (24)	1.11 ± 0.06	1.18 ± 0.03	0.09
Neopterin (nmol/L)	B < W (24)	4.09 ± 0.26	4.53 ± 0.13	0.07
IgG (mg/dl)	B > W (25)	1496 ± 45	1213 ± 25	<0.0001
IgM (mg/dl)	B = W (25)	125 ± 9	180 ± 10	0.001
IgA (mg/dl)	B = W (25)	185 ± 13	181 ± 8	>0.1
		Girls	Boys	*P* value[b]
sIL-2R	F < M (23)	513 ± 37	605 ± 45	0.002
B2M	F < M (24)	1.07 ± 0.04	1.26 ± 0.04	0.0003
Neopterin	F = M (24)	4.39 ± 0.15	4.66 ± 0.18	>0.1
IgG (mg/dl)	F = M[c]	1256 ± 32	1300 ± 36	>0.1
IgM (mg/dl)	F > M[c]	187 ± 11	146 ± 10	0.03
IgA (mg/dl)	F = M[c]	184 ± 10	180 ± 9	>0.1

[a] By multiple linear regression, controlling for gender and age.
[b] By multiple linear regression, controlling for race and age.
[c] From reanalysis of data in adult population (not published).

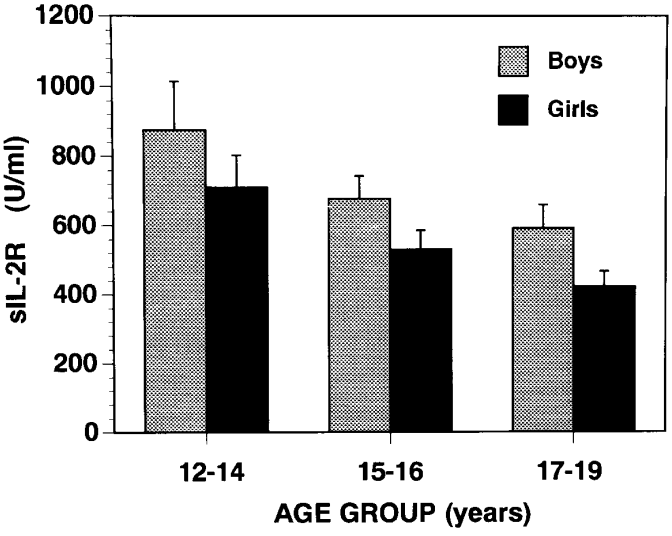

FIG. 1. Serum sIL-2R levels by age group and sex: geometric means and SE.

significantly higher sIL-2R and B2M levels and lower IgM levels than girls.

Age-Related Changes

Significant differences among age groups were observed for sIL-2R for both boys and girls (Fig. 1). Stratification by race showed that serum sIL-2R level was associated with age among whites, but not among blacks (Fig. 2). To assess the consistency of age-related changes, values of these three markers among adolescents were compared with age-stratified values in the

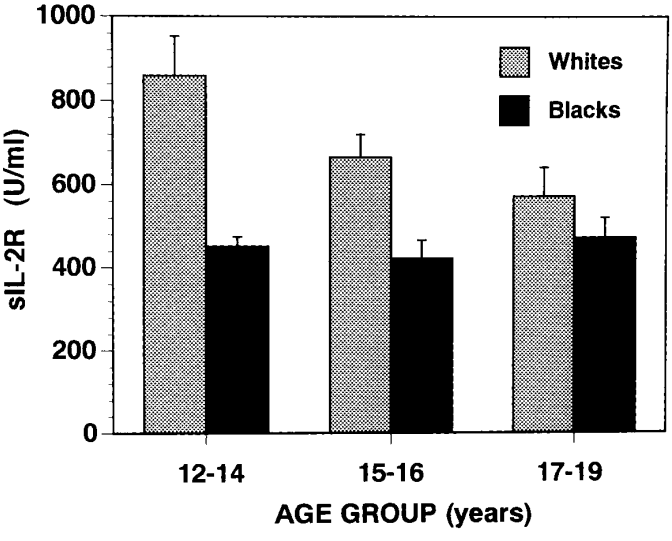

FIG. 2. Serum sIL-2R levels by age group and race: geometric means and SE.

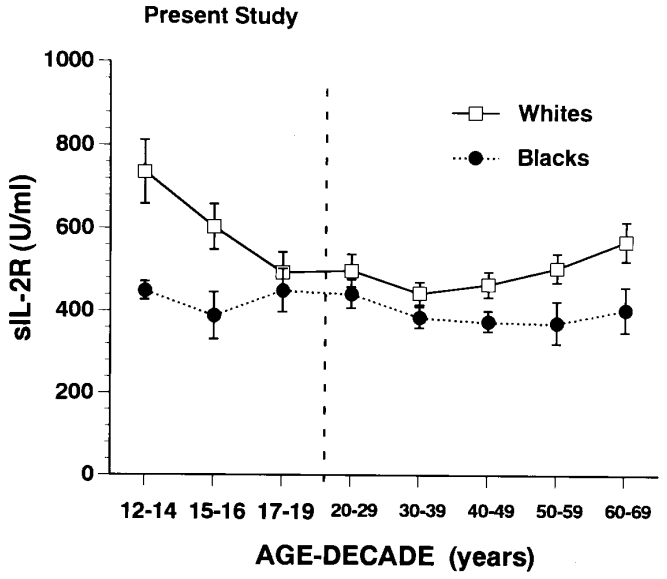

FIG. 3. Influence of age and race on serum sIL-2R. Values for adolescents are compared to values among adults ages 20–69 years (from (23)): geometric means and SE. The vertical dashed line separates the new data presented in this paper (left of the line) from previously published data in adults (right of the line).

previously analyzed 20- to 69-year-old population from which the adolescent subjects were recruited (Figs. 3–5). Because there was no significant age effect on B2M or neopterin level over the age range of 12–19

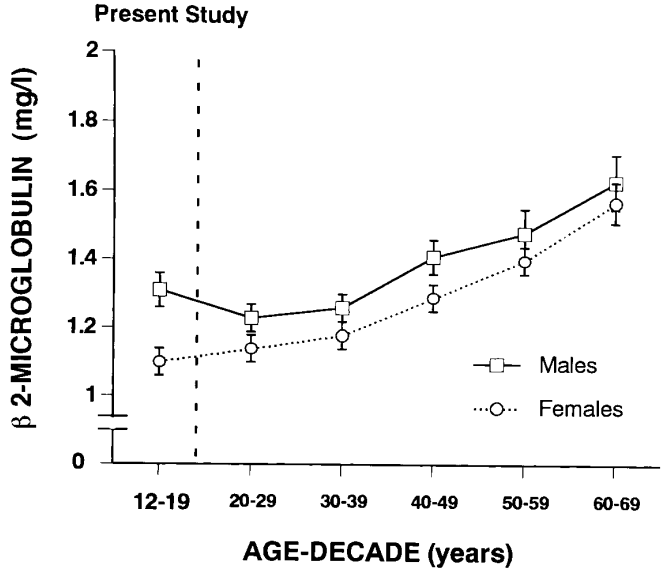

FIG. 4. Influence of age and gender on serum B2M. Values for adolescents are compared to values among adults ages 20–69 years (from (24)): geometric means and SE. The vertical dashed line separates the new data presented in this paper (left of the line) from previously published data in adults (right of the line).

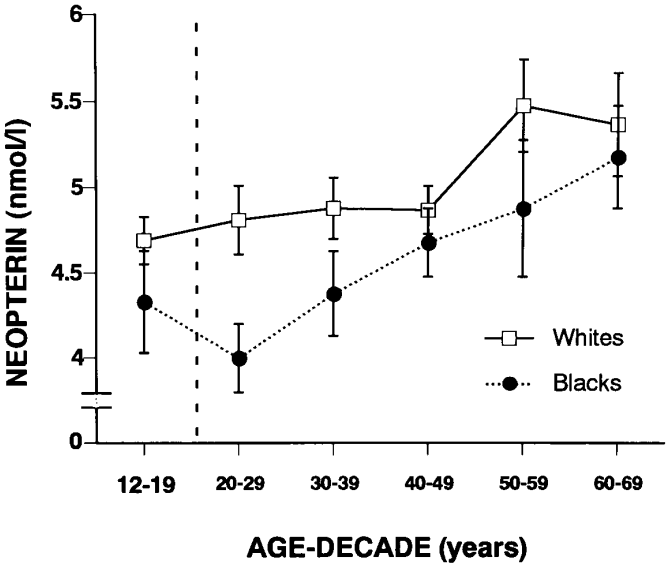

FIG. 5. Influence of age and race on serum neopterin. Values for adolescents are compared to values among adults ages 20–69 years (from (24)): geometric means and SE. The vertical dashed line separates the new data presented in this paper (left of the line) from previously published data in adults (right of the line).

years included in this analysis, a single mean value for ages 12–19 is presented in Figs. 4 and 5. There were no significant age effects observed for immunoglobulins.

DISCUSSION

In this report, we present data on serum levels of several key biomarkers in a healthy white and African-American population of adolescents. A number of important observations emerged from these analyses. First, the decline in sIL-2R level which begins in infancy among whites (29) continues through the early teens, reaching "adult" levels by age 17–19. Little is known about sIL-2R levels in blacks. The present analysis, combined with our previously published data in adults (23), suggests that blacks have consistently lower levels of serum sIL-2R *and* do not exhibit the same age-related changes in sIL-2R level as whites. As in adults, males had higher sIL-2R levels than females. Our previous analysis of healthy adults ages 20–69 years showed that: (1) both neopterin and B2M levels increased with age and were higher among whites than blacks and (2) gender differences were noted for B2M, but not for neopterin values (24). In a related study, Komp *et al.* (30) measured sIL-2R in the sera of 122 normal children (race was not specified). The highest values (1339 ± 498 U/ml) were recorded during 9.5–19.5 months of age, gradually declining to their adult level of 273 ± 101 U/ml. From these results they emphasized the necessity of applying age-specific controls when evaluating the elevation of serum sIL-2R level due to immune activation. In that study only 16 adolescents were recruited in the category of 10+ years, with a mean level of 340 ± 130 U/ml sIL-2R.

In the present study, no significant age effect was observed for B2M or neopterin over the narrow age range of 12–19 years, although race and gender effects were observed, similar to those seen in adults. Reibnegger *et al.* (31) measured serum neopterin in 53 healthy young women ages 21–34 years and 51 healthy elderly women ages 75–91 years and observed significantly higher levels of neopterin in the elderly. Currie *et al.* (32) measured serum neopterin level in a cohort of 282 elderly subjects between the ages of 70 and 79 and found no differences in neopterin level on the basis of gender or race.

Several previous studies using different age groups have suggested that both race and gender may have important influences on serum immunoglobulin levels (33–40). Studies of African infants (36) and Chinese children (37) found higher serum IgM levels in females. We found similarly higher IgM levels in girls than in boys. For IgG, significantly higher levels of IgG among girls were observed in Africans, though there were no significant differences between males and females in Chinese children. The current study confirms these observations of higher immunoglobulin levels, particularly IgG, among blacks compared to whites (25, 39, 40). The present study suggests that these differences, as with sIL-2R and neopterin, are well established by early adolescence.

It is important to note that children in this analysis had been recruited from households participating in a study of healthy adults. While the adult and teenage groups are not strictly comparable, they were recruited from a common geographic base, with a similar racial and sociodemographic distribution. Thus, comparisons between the previously published data from the adults and the new data from the children seem appropriate. The care taken to confirm the health status of the adolescents participating in the study supports the generalizability of these results to other populations of healthy teenagers. These data provide important baseline information for clinical and epidemiologic investigators and emphasize the importance of considering demographic characteristics for normative adjustments in analyses utilizing these biomarkers.

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